



## Chromosomal imbalance letter

Deletion of the *RMGA* and *CHD2* genes in a child with epilepsy and mental deficiency

Leonardo P. Capelli<sup>a</sup>, Ana C.V. Krepischi<sup>a</sup>, Juliana Gurgel-Giannetti<sup>b</sup>, Mirian Fabiola S. Mendes<sup>c</sup>, Tatiane Rodrigues<sup>d</sup>, Monica C. Varela<sup>d</sup>, Célia P. Koiffmann<sup>d</sup>, Carla Rosenberg<sup>d,\*</sup>

<sup>a</sup> A. C. Camargo Cancer Hospital, São Paulo, Brazil

<sup>b</sup> Pediatric Department, Federal University of Minas Gerais, Brazil

<sup>c</sup> Madre Teresa Hospital, Belo Horizonte, Brazil

<sup>d</sup> Department of Genetics and Evolutionary Biology, Institute of Biosciences, University of São Paulo, Brazil

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## ABSTRACT

We describe a novel chromosome microdeletion at 15q26.1 detected by oligo-array-CGH in a 6-year-old girl presenting with global development delay, epilepsy, autistic behavior and facial dysmorphisms. Although these features are often present in Angelman syndrome, no alterations were present in the methylation pattern of the *Prader-Willi–Angelman critical region*. The deletion encompasses only 2 genes: *CHD2*, which is part of a gene family already involved in CHARGE syndrome, and *RGMA* which exerts a negative control on axon growth. Deletion of either or both genes could cause the phenotype of this patient. These results provide a further chromosome region requiring evaluation in patients presenting Angelman features.

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## 1. Methods of detection

## 1.1. Cytogenetics

Chromosome analysis of peripheral blood lymphocytes by G-banding at the 550 or higher banding level showed a normal 46,XX karyotype.

## 1.2. Array-CGH

The procedure was performed using a whole-genome oligo-array platform containing 180,000 oligonucleotides (BlueGnome, design 24585). The labeling, hybridization, and post-hybridization washing stages were performed according to Agilent's protocol. Scanned images of the arrays were processed using Agilent Feature Extraction software, and analyzed with Genomic Workbench software (both from Agilent Technologies), with the statistical algorithm ADM-2, and sensitivity threshold 6.7. At least 3 consecutive oligonucleotides had to exhibit aberrant  $\log_2$  ratios of the same sign to be recognized by the software.

## 1.3. Chromosome alteration

The analysis revealed a 511 kb microdeletion at 15q26.1 (Fig. 1a), ranging from genomic position 93,412,860 to 93,923,856 (GRCh37/hg 19). The deleted segment encompasses only 2 genes, *CHD2* and *RGMA*. Array-CGH analysis of the parents showed that the microdeletion in the child was *de novo*.

1.4. Methylation analysis of the *Prader-Willi–Angelman critical region* (PWACR)

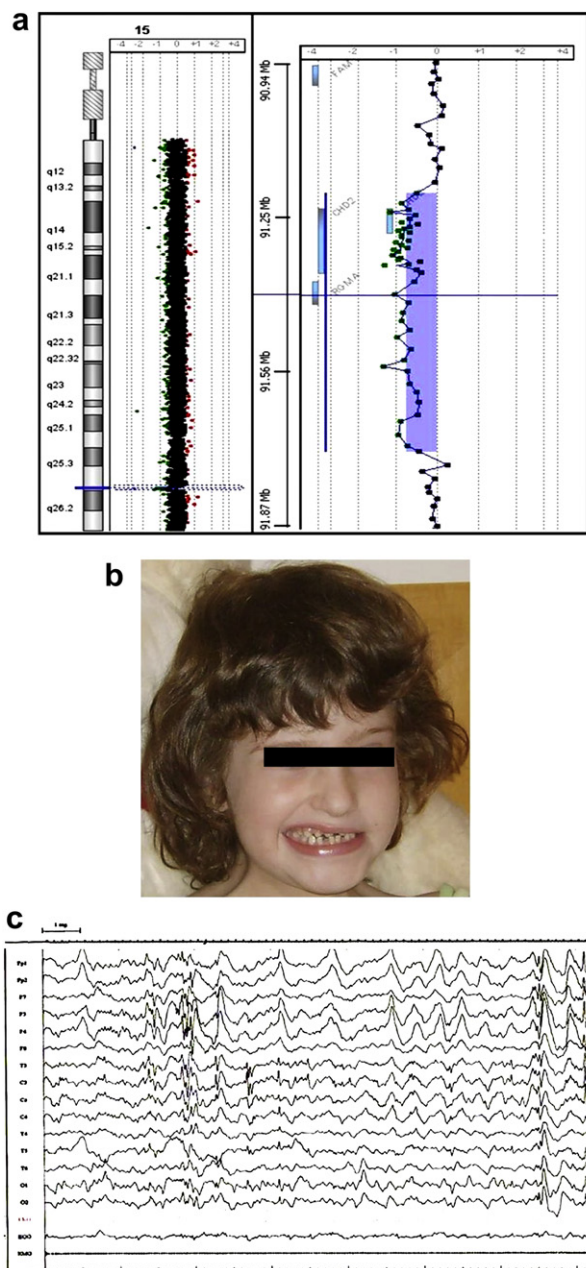
The methylation status of the PWACR was based on the pattern obtained from exon 1 of the *SNURF–SNRPN* gene after PCR amplification of bisulfite-modified DNA. This methodology is based on primers specific to paternal and maternal alleles that amplify fragments of 221 and 313 bp, respectively, as described by [1]. No alterations in the methylation pattern were detected suggesting that the PWACR was not involved in the phenotype of this patient.

## 2. Clinical description

The proband was the third child of non-consanguineous parents, born after an uneventful pregnancy. She was referred for genetic evaluation at the age of six years and presented global development delay, epilepsy, autistic behavior, and facial

\* Corresponding author. Departamento de Genética e Biologia Evolutiva, Instituto de Biociências, Universidade de São Paulo, Rua do Matão, 277, Sala 337, CEP 05508-090, São Paulo, Brazil. Tel.: +55 11 3091 7590; fax: +55 11 3091 7553.

E-mail address: [carlarosenberg@uol.com.br](mailto:carlarosenberg@uol.com.br) (C. Rosenberg).



**Fig. 1.** (a) Copy number determination of chromosome 15 of our patient obtained by oligo-array-based comparative genomic hybridization (180K platform). The ~500 kb microdeletion at 15q26.1 is indicated by the blue-dashed line on the left and shown in detail on the right. The two genes encompassed by the deletion, *RGMA* and *CHD2*, are represented by light blue bars; (b) Frontal view of the patient with microdeletion at 15q26.1; (c) electroencephalogram showing generalized spike-waves associated with focal discharges, a distinctive pattern displayed by AS patients.

dysmorphisms. Physical examination revealed weight, length and cranium circumference at the 50th, 75th and 10th percentiles, respectively. The skin, hair and eyes were light in color. Facial dysmorphisms included prognathia, wide mouth, short and widely spaced teeth, and non-paralytic strabismus. Her facial gestalt was suggestive of AS (Fig. 1b). Behavior disturbances included high excitability and short attention span. Neurological examination revealed severe speech impairment with minimal use of words (around 10–20); in addition, the patient displayed gait ataxia accompanied by uplifted arms, and hand flapping. There was slight hypotonia with normal muscle strength, and hyperactive deep

tendon reflexes without the Babinski sign. Seizures started at the age of 24 months, and were partially controlled with valproic acid. Brain MRI revealed no severe abnormalities and showed generalized spike-waves associated with focal discharges. The focal epileptiform waves were mainly localized to the rolandic-parietal region, especially in the left hemisphere (Fig. 1c), and differed from the three EEG patterns (delta pattern, theta pattern, and posterior discharges) suggestive of AS [2].

Because the patient's phenotype partially overlapped with Angelman syndrome, we compared her clinical features with those listed in the Diagnostic Criteria for AS [3], in which the features associated with AS are divided into 3 categories, namely: **consistent** (present in 100% of AS patients), **frequent** (present in 80% of AS patients), and **associated** (present in 20–80% of AS patients). Our patient exhibited:

- All 4 **consistent** features (severe development delay; gait ataxia; easily excitable personality with uplifted hand flapping; speech impairment with minimal use of words).
- 2 out of 3 **frequent** features (disproportionate growth in head circumference, which often but not always results in microcephaly; seizures with onset <3 years of age).
- 6 out of 21 **associated** features (prognathia; wide mouth, widely spaced teeth; strabismus; hypopigmented skin and light hair and eyes color; hyperactive lower extremity deep tendon reflexes).

### 3. Discussion

According to the Diagnostic Criteria for AS [3], the patient described here fulfills all obligatory and a considerable proportion of the frequent and associated features of AS. However, the results of her electroencephalograms were not typical of AS and, although she presents a disproportionate reduction in size in head circumference relative to normal, she does not present microcephaly. Because microcephaly is a frequent but not obligatory component of Angelman Syndrome, and EEG was only performed later, we initially could not exclude the diagnosis of AS, and methylation studies were performed; as stated these were negative for the methylation pattern in the PWACR expected for Angelman syndrome.

Other critical chromosomal abnormalities have been described in patients where clinical signs were initially suggestive of AS, including 17q21.3 [4] and 22q13.3 [5]. The deletion described here appears to be the smallest reported in region 15q26.1 and encompasses only two genes, *RGMA* (repulsive guidance molecule, member A), and *CHD2* (chromodomain helicase DNA binding protein 2). No normal copy number variation overlapping these 2 genes has been documented (Database of Genomic Variants - <http://projects.tcag.ca/variation/> freeze in November 2010). On the other hand, much larger deletions encompassing *CHD2* and *RGMA* (3.5 Mb–19 Mb) have been described in the DECIPHER (Database of Chromosomal Imbalance and Phenotype in Humans Using Ensembl Resources - <http://decipher.sanger.ac.uk/>) [6], and very little phenotypic data is provided, impairing comparison with our patient. In particular, one article mapped deletions to 15q26 in patients with congenital diaphragmatic hernia encompassing a minimum region of ~5 Mb and containing 4 genes, including *CHD2* and *RGMA* [7]. However, the fact that our patient did not exhibit congenital diaphragmatic hernia suggests that these two genes are unlikely candidates for this feature.

*CHD2* is part of a gene family involved in the negative regulation of gene expression through chromatin remodeling and assembly (<http://www.genecards.org/>), and alterations in genes of this family have already been implicated in human diseases, such as *CHD7*, in CHARGE syndrome (review in [8]). Two other papers reported

mutation and disruption of *CHD2* in murine models, associated with respectively complex renal phenotype [9] and scoliosis [10]. Each of these malformations was observed in separate mouse models indicating that, at least in mice, other factors regulate the effect of *CHD2* alterations. Our patient presented neither renal malformations nor scoliosis.

The other gene, *RGMA*, acts in the negative control of axon growth (<http://www.genecards.org/>). Because of its role in the central nervous system, deletion of *RGMA* appears as candidate for both the mental deficiency and seizures presented by the girl reported here. These results suggest that haploinsufficiency of the *RGMA*, and/or *CHD2* genes results in features suggestive of AS, and provide a further region to be evaluated in the differential diagnosis of Angelman-like phenotypes.

#### 4. Dissemination of information

The patient's clinical and molecular data have been deposited under patient number 249888 in DECIPHER (Database of Chromosomal Imbalances and Phenotype in Humans using Ensembl Resources; – <http://www.sanger.ac.uk/PostGenomics/decipher>).

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#### Web resources

- [11] DECIPHER, <http://www.sanger.ac.uk/PostGenomics/decipher>.
- [12] Database of Genomic Variants (DGV), <http://projects.tcag.ca/variation/>.
- [13] Genecards, <http://www.genecards.org/>.